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A-to-I RNA Editing and Human Disease

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Abstract

The post-transcriptional modification of mammalian transcripts by A-to-I RNA editing has been recognized as an important mechanism for the generation of molecular diversity and also regulates protein function through recoding of genomic information. As the molecular players of editing are characterized and an increasing number of genes become identified that are subject to A-to-I modification, the potential impact of editing on the etiology or progression of human diseases is realized. Here we review the recent knowledge on where disturbances in A-to-I RNA editing have been correlated with human disease phenotypes.

Keywords

ADAR; RNA editing; ALS; DSH; depression; epilepsy; serotonin receptor; glutamate receptor

1. ADAR and A-to-I RNA editing

The complexity of higher organisms is based on the number of different gene products available for structural, enzymatic and regulatory functions. With a rather limited set of genes available in the genomes of even the most highly evolved species, mechanisms controlling gene utilization play a major role in the creation of proteomic and phenotypic diversity. Several post-transcriptional and post-translational mechanisms have been identified that lead to the production of multiple gene products from a single gene. The alternative splicing of pre-mRNAs is a particularly frequent event, estimated to affect more than 70% of mammalian primary transcripts [1,2]. Another post-transcriptional processing pathway that appears to be widespread in mammals is A-to-I RNA editing where individual adenosine (A) bases in pre-mRNA are modified to yield inosine (I). A-to-I RNA editing is catalyzed by ADAR (adenosine deaminase acting on RNA) [3-6]. Three ADAR gene family members (ADAR1-3) have been identified in vertebrate (Fig. 1A) [7,8]. ADAR1 and ADAR2 are detected in many tissues, whereas ADAR3 is expressed specifically in brain. ADAR1 protein is synthesized in two forms as a result of alternative splicing. The ADAR1 mRNA transcribed from an IFN-inducible promoter [9] directs translation of a full-length 150-kDa form of ADAR1 protein (p150). The two other ADAR1 mRNAs, transcribed from constitutive promoters, direct synthesis of a shorter 110-kDa ADAR1 protein (p110) initiated from a downstream Met codon (Fig. 1B). The IFN-inducible p150 protein is detected mainly in the cytoplasm, whereas the constitutively expressed p110 protein is present exclusively in the nucleus [9]. A single ADAR2-like gene, *dADAR*, is present in *D. melanogaster* [10], whereas two less conserved genes, *c.e.ADA1* and *c.e.ADA2* exist in *C. elegans* [11] (Fig. 1A). The sequences of *c.e.ADA1* and *c.e.ADA2* differ

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significantly from the three mammalian ADAR genes [11-13]. No ADAR gene has been identified in the genomes of plants, fungi and yeast.

Since inosine acts as guanosine during translation, A-to-I conversion in coding sequences leads to amino acid changes and often entails changes in protein function. The power of RNA editing in the generation of protein diversity lies in the fact that usually both the edited and unedited versions of the protein are co-expressed in the same cell and the ratio between the unedited and edited variants can be regulated in a cell type-specific or time-dependent manner. In addition to these cases where the editing occurs in coding regions of gene transcripts, A-to-I editing events have also been detected in noncoding regions of cellular genes [14,15] and within viral RNAs after infection [16-18]. In one case, an RNA editing enzyme edits its own pre-mRNA creating a new splice site that leads to alternative splicing [15,19]. Recently, thousands of editing sites have been mapped to Alu repeat elements within human pre-mRNAs [20-23], including some examples that could lead to the alteration or creation of splice sites by A-to-I modification [20]. The editing events seen in 5′- and 3′- untranslated regions and the intronic sequences 5 might point toward yet unexplored roles of editing in regulating the transport, stability, or further processing of RNAs as well as silencing of retrotransposons.

In mammals, as well as flies and worms, A-to-I RNA editing regulates important functional properties of neurotransmitter receptors in the central nervous system [11-13,24-28]. In particular 5 the glutamate receptor subunit GluR-2 undergoes almost quantitative editing (>99.9 %) at one position (the Q/R-site), which represents the molecular determinant for low Ca^{2+}-permeability of the ion channel [26]. In addition, the Q/R-site regulates the intracellular trafficking and tetramerization of the receptor protein [27,28]. Transgenic mice with even slightly reduced GluR-2 editing suffer severe epileptic seizures and die within 2 weeks of age [29]. This phenotype was a consequence of the increased Ca^{2+}-permeability of the underedited glutamate receptors [29]. The same phenotype resulted when the editing enzyme ADAR2 was inactivated in mice, due to a dramatic reduction in Q/R-site editing [13]. Other neuronal genes affected by A-to-I RNA editing include the glutamate receptor subunits GluR-3, -4, -5, and -6 where RNA editing regulates gating and kinetic properties of the ion channels [30,31], the 5-HT_{2C} serotonin receptor subtype where editing is known to regulate G-protein coupling functions of the receptor [32], and the K(V)1.1 potassium channel where editing regulates channel inactivation [33].

Since RNA editing serves to fine-regulate neurotransmission and is involved in the evolution of higher functions, it is naturally anticipated that malfunction of the process would lead to human diseases of learning, memory, language, thinking, and behavior. In the last several years, diseases of the central nervous system, such as depression, epilepsy, schizophrenia and amyotrophic lateral sclerosis (ALS) [34] have indeed been linked to a deregulation of RNA editing. In addition, the genetic linkage map of a human pigmentation disease led to identification of ADAR1 as the responsible gene.

2. Dyschromatosis symmetrica hereditaria

Dyschromatosis symmetrica hereditaria (DSH) is a pigmenting genodermatosis with an autosomal dominant inheritance [11-13]. In two independent studies, one based on several affected Japanese families [35] and one using Chinese pedigrees [36], the locus for DSH was recently mapped to the ADAR1 gene on chromosome 1q21.3 through linkage analysis followed by mutational scanning/SNP mapping. An initial set of 11 independent mutations were identified (4 Japanese and 8 Chinese pedigrees) all co-segregating with the DSH phenotype. Since then, 21 additional ADAR1 mutations (heterozygous) were identified in DSH patients [37-44]. Figure 2 lists all known mutations with their predicted consequences on ADAR1 expression, which consists of 12 missense, 9 frameshift, 8 nonsense, and 3 splice site (IV2,

IV8 and IV12) mutations. Among 32 mutations identified in total, the H216fs mutation (Fig. 2) is specifically interesting, since this frame shift mutation is located upstream of the methionine codon 296, which is used as the initiation codon for translation of the p110 form of ADAR1 [45]. Thus, no p150 form ADAR1 is synthesized from the mutated allele, but there should be no problem to synthesize p110 proteins in the patients carrying this particular mutation (Fig. 1B). This mutation is clearly linked to the DSH phenotype, indicating that the dosage of functional p150 proteins is a dominant denominator of DSH. It has been recently reported that the p150 form of ADAR1 is involved in antiapoptotic pathways [46]. Furthermore, p150 appears to regulate cellular RNAi efficacy by sequestering siRNA [47]. It is of special interest to find out whether these ADAR1 functions specific to the p150 isoform, especially its function in the regulation of RNAi, have any causative relevance to DSH pathogenesis.

Regardless of the involvement of p150 or p110, the observed link of ADAR1 gene mutations and DSH raises several questions with regard to the disease mechanism and the role of different ADAR1 mutant proteins. For example, there seems to be no correlation between genotype and phenotype as carriers with the same mutation show distinct clinical manifestations of the disorder indicating the contribution of environmental factors on disease development and progression [36]. Furthermore, in some of the Chinese pedigrees the penetrance of the ADAR1 mutation was not 100%. Since in those patients no other ADAR1 mutations were detected it is not clear if in those cases the inherited mutation has a causative role in disease development.

The fact that DSH is inherited in a dominant fashion indicates a gain-of-function disease mechanism or a dominant negative effect of the mutant proteins on normal ADAR1 function. The finding that formation of a homodimer complex is essential for enzymatic activities of mammalian ADAR1 and ADAR2 [48] as well as Drosophila dADAR [49] certainly supports the latter possibility. Finally, it is noteworthy that in mice heterozygous for an *ADAR1 null* mutation no phenotype is discernible [46,50], whereas homozygous ADAR1 knock-out animals die during early embryogenesis [46,50]. No pigmentation abnormality is detected with limbs of *ADAR1*$^{+/-}$ mice (K. Nishikura, unpublished results). It will be interesting to see if an ADAR1-specific RNA editing target(s) will emerge in the future that can explain the observed DSH phenotype and disease mechanism.

3. Amyotrophic lateral sclerosis

ALS is a progressive neurodegenerative disease that affects approximately 1 in 2000 people over their lifetime [51]. Usually fatal within 1-5 years after onset, the causes for most cases of ALS (90-95% of all cases with sporadic onset) are unknown. In ALS a specific subset of neurons (motor neurons) degenerates causing the progressive symptoms associated with ALS: muscle weakness, muscle atrophy, spasticity and eventually paralysis and respiratory failure. The unusual asymmetry of motor neurons with axons of up to 1 meter in length makes them particularly vulnerable for disturbances in axonal transport and metabolic defects.

Studies on familial cases of ALS which make up 5-10% of cases have implicated several genes and pathways in the pathology of the disease. In 15-20% of familial ALS mutations in the gene of radical scavenger SOD1 (converts superoxide to water and hydrogen peroxide) are associated with ALS possibly through a gain-of-function mechanism. Deposits of SOD1 and other proteins in motor neurons that are detectable before onset of clinical symptoms are believed to be caused by misfolded SOD1 and proteasome dysfunction due to aggregated, mutant SOD1. Both in sporadic and familial ALS, accumulation of neurofilament proteins in axons of motor neurons is observed, but as for other types of protein deposits associated with several neurodegenerative diseases (such as Alzheimer's and Parkinson Disease) it is not clear if the deposits are causative for the disease or might serve a protective function.

Glutamate excitotoxicity appears to contribute to the pathology of sporadic ALS: glutamate levels were increased in 40% of sporadic ALS cases, suggesting that prolonged activation of glutamate gated ion channels might lead to excessive Ca^{2+} influx and neuronal death [52]. The cause for the observed increase in synaptic glutamate could be traced to a selective loss in activity of EAAT2 (excitatory amino acid transporter 2), a glutamate transporter located on astrocytes that quickly clears the synaptic cleft of glutamate after excretion [53].

The contribution of excessive Ca^{2+} influx through glutamate receptors to the death of motor neurons is at the heart of the recent suggestion that RNA editing might play a role in ALS. Editing of the Q/R position in GluR-2 is the molecular determinant for the observed low Ca^{2+}-permeability of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-type glutamate receptors in most neurons. A decrease or loss of RNA editing function specifically in motor neurons could lead to AMPA-channels highly permeable to Ca^{2+}, mimicking or exacerbating the overexcitation of glutamate receptors due to EAAT loss. This is exactly what Kwak and colleagues found when they analyzed the RNA editing status of GluR-2 in spinal motor neurons from ALS patients in comparison with normal controls [34, 54-56]. In the diseased neurons editing of the GluR-2 Q/R position was severely decreased in most samples (ranging from 62-100%) whereas all control cells showed 100% editing [55]. The deficiency in RNA editing of the GluR-2 Q/R position is not detected in motor neurons of rats transgenic for mutant human SOD1 (an animal model for families with ALS) or in those of patients with spinal and bulbar muscular atrophy, a non-ALS motor neuron disease [57]. These findings indicate that abnormal editing may be a contributory cause of neuronal death specifically in sporadic ALS [56]. It is not known when during disease progression this impairment in editing arises or if it might be a causative event during the onset of ALS. Furthermore, the editing deficiency is seen only in spinal motor neurons, whereas upper motor neurons, which also degenerate in ALS, showed unaltered editing levels of GluR-2. This means that a defect in Q/R site editing cannot be the sole mechanism responsible for the demise of the motor neurons. Maybe a mouse model with inducible motor neuron specific RNA editing deficiency will be able to resolve this issue.

Additional evidence for a contribution of Ca^{2+}-permeable AMPA-receptors to motor neuron degeneration comes from a recent genetic mouse model [45]. The expression of AMPA-receptors that harbor GluR-2 subunits with the residue N at the Q/R position results in channels with modestly increased Ca^{2+}-permeability (with no substantial effect on overall conductance [58]) and the mice develop late-onset motor neuron disease resembling the pathological changes observed in sporadic ALS. As discussed below, substantial increase in Ca^{2+}-permeability of AMPA receptors in principal neurons leads to early lethality precluding the observation of late-onset degenerative disease. Furthermore, in the background of the SOD1 G93A mutation, which has been linked to cases of familial ALS [51], the GluR-2(N) mutation accelerates disease progression and decreases survival [59]. Figure 3 illustrates the current knowledge of how RNA editing deficiency specifically in motor neurons might contribute to motor neuron death in ALS.

4. Epilepsy

Epilepsy is one of the most common neurological disorders characterized by recurrent, unprovoked seizures [for review see [60]]. The causes for epilepsy as well as the symptoms associated with a seizure can be divers and depend on the normal functional roles of the affected neuronal subpopulation. All epileptic phenotypes share the common feature of neuronal hyperexcitability that occasionally leads to seizures.

Due to the central function of AMPA-type glutamate receptors in fast excitatory neurotransmission, they also have been recognized to play a prominent role in the development,

expression and spread of seizures. In various instances changes in AMPA receptor subunit expression, channel density or posttranscriptional regulation have been observed in human epilepsy or animal seizure models [61]. Generally, CNS injury, such as stroke or traumatic brain injury can lead to secondary neuronal death due to glutamate exitotoxicity, which causes an irreversible elevation in intracellular Ca^{2+}ions. The Ca^{2+} hypothesis of epileptogenesis proposes that neurons that are exposed to extended but non-lethal levels of elevated Ca^{2+} undergo plasticity changes that result in acquired epilepsy [60].

Several lines of evidence point towards a connection between changes in glutamate receptor editing and seizure vulnerability. The epileptic phenotypes seen in GluR-2 editing deficient mice [13,29] are most profound and the severity of seizure activity correlates directly with degree of impairment in editing of the Q/R-position [58]. The molecular mechanism for epileptogenesis in these transgenic mice has not been elucidated, but it is likely that the changes in AMPA-channel properties due to the expression of GluR-2(Q) are the cause for the observed seizure phenotype. This is suggested by the fact that the phenotype can be rescued by pre-editing GluR-2 messages through genomic mutation [13]. The epileptic phenotype of the described editing deficient mouse models could be the result of a neurodevelopmental defect instead of being a direct consequence of altered AMPA-receptor function in the adult brain. This issue was recently addressed in a conditional mouse mutant where GluR-2 editing is inactivated postnatally in selected forebrain regions [62]. In support of the notion that unedited GluR-2 directly causes neuron hyperexcitability it was observed that also in adult mice editing deficiency induced seizures with similarity to human temporal lobe epilepsy [62].

A critical remaining question is how changes in GluR-2 Q/R site editing lead to hyperexcitability and epileptic seizures. The results from the various mouse models that alter AMPA receptor function by either downregulating or eliminating GluR-2 expression or by inhibiting GluR-2 editing show that expression of unedited GluR-2(Q) is a necessary requirement for seizure generation. In contrast, elimination of GluR-2 expression, which also results in Ca^{2+}-permeable AMPA receptors, does not precipitate an epileptic phenotype in mice. These differences are likely due to the fact that the nature of the Q/R site residue determines not only the AMPA receptor Ca^{2+}-permeability, but also regulates gating kinetics, channel conductance, and channel assembly, as well as channel trafficking [27,28]. Epilepsies associated with human malignant brain tumors (glioblastoma multiforme) [reviewed in [63]] might also be linked to a decrease in GluR-2 Q/R site editing [64]. RNA editing of the GluR-2 R/G site, as well as the alternative splicing of 5-HT_{2C} receptor pre-mRNA, which is co-regulated by editing, were also altered [64]. ADAR2 activity was found to be reduced in tissue specimen from glioblastoma patients compared to white matter controls [64].

An editing deficiency at the Q/R editing site of the kainate-specific glutamate receptor subunit GluR-6 has been linked to seizure vulnerability in mice [65]. The deletion of the editing site complementary sequence (ECS) in the mouse GluR-6 gene, which results in a complete loss of editing at the Q/R position, did not give rise to spontaneous seizures, and there was no change in the time to seizure onset after kainate injection, but rather increased seizure vulnerability in GluR-6 ECS knock-out mice. Vulnerability to kainate-induced seizures was inversely correlated with the extent of GluR-6 Q/R-site editing [65]. Kainate-induced seizures in mice are a model of temporal lobe epilepsy in humans [66,67]. However, analysis of tissue specimen from patients with temporal lobe epilepsy did not show a decrease in editing efficiency at any of the GluR sites [68].

5. Depression and schizophrenia

No specific gene has been definitively identified for any major psychiatric disorder, including major depression and schizophrenia [69]. While this might in part be due to poor study design

and the complexity of mental disorders, epigenetic factors that modify gene expression have been indicated as another potentially important issue. Because epigenetic modifications are essential mechanisms for most genes to adapt functionality in response to a variable environment, an inadequate epigenetic modification of the genes could be a causative mechanism for inducing mental disorders.

Members of the serotonin (5-hydroxytryptamine or 5-HT) receptor gene family are believed to play important roles in physiological and behavioral processes such as circadian rhythms, emotion, appetite and sexual behavior. Furthermore, 5-HT receptors may have a causative relevance to human mental abnormalities and pathology, including major depression, schizophrenia, anxiety, migraine, and substance dependency [70]. Among at least 14 distinct subtypes of 5-HT receptors (5-HTRs) expressed within the central nervous system [71], the 5-HT_{2C}R is the only known G-protein coupled receptor whose mRNA undergoes post-transcriptional editing, a major epigenetic mechanism, to yield different receptor isoforms in humans and rodents [72].

Editing in the *5-HT_{2C}R* pre-mRNA takes place at five positions in exon 5 (Fig. 4A). The receptor function is modulated by RNA editing through three distinct mechanisms. First, editing of five sites (A-E sites) situated within the second intracellular loop region changes the genomically-encoded amino acids Ile, Asp, and Ile at positions 156, 158, and 160, respectively, resulting in alteration of G protein-coupling efficacy (Fig. 4B). The fully edited isoform (5-HT_{2C}R-VGV) profoundly reduces 5-HT potency, G-protein coupling and agonist binding, compared to the unedited protein isoform (5-HT_{2C}R-INI) (Fig. 4B) [72-76]. Second, the editing pattern controls the amount of the *5-HT_{2C}R* mRNA that leads to the expression of full-length protein through the modulation of alternative splice site selection [76,77]. Among three alternative splice donor sites (GU1 to GU3; Fig. 4C), GU2 is the only site that forms the mature mRNA to produce the functional, full-length 5-HT_{2C}R protein. Unedited pre-mRNAs tend to be spliced at the GU1 site, resulting in the truncated, non-functional protein if translated [76,77]. However, most pre-mRNAs edited at more than one position are spliced at GU2 [77]. Thus, when editing is inefficient, increased splicing at GU1 may act as a control mechanism to decrease biosynthesis of the 5-HT_{2C}R-INI and thereby limit serotonin response. Third, RNA editing controls the ultimate physiological output of constitutively active receptors by affecting the cell surface expression of the 5-HT_{2C}R. The 5-HT_{2C}R-VGV, which displays the lowest level of constitutive activity, is fully expressed at the cell surface under basal conditions and is rapidly internalized in the presence of agonist [78]. In contrast, the 5-HT_{2C}R-INI is constitutively internalized and accumulates in endosomes [78].

Associations between major psychiatric disorders and the pattern of RNA editing at 5 editing sites of the 5-HT_{2C}R have been investigated in recent years (Table 1). No alteration of the editing pattern was found in three independent studies conducted regarding schizophrenia [79-81]. One group claimed that the expression level of the 5-HT_{2C}R-INI was significantly increased [82], however, this discrepancy could be due to small sample size, different methodologies, and the medication history of investigated subjects. On the other hand, multiple groups reported an increased RNA editing efficiency at the A site in brains of depressed suicide victims [80,81]. Moreover, Gurevich et al. reported that E site editing is significantly increased as well in individuals with major depression [83]. This alteration is also observed in a rat model of depression and can be reverted through treatment with the serotonin-selective reuptake blocker fluoxetine [84]. In addition, mice chronically treated with fluoxetine also exhibit decreased E site editing [85], suggesting that E site editing might be associated with the pathophysiological status of major depression.

It has been revealed that the pattern of 5-HT_{2C}R editing is significantly different among inbred strains of mice [86]. In C57BL/6 and 129SV, more than 80% of forebrain neocortical *5-HT_{2C}R*

mRNAs are edited at A, B, and D sites or A, B, C, and D sites. C57BL/6 mice show no alteration of the editing pattern against acute stress or chronic treatment of fluoxetine. In contrast, in the BALB/c strain, about 80% of *5-HT$_{2C}$R* mRNAs are not edited at all and encode receptors with the highest constitutive activity and the highest agonist affinity and potency. However, exposure of BALB/c mice to acute stress or chronic treatment with fluoxetine elicit significant increases of RNA editing at sites A to D [86]. Interestingly, BALB/c mice have a deficiency in the 5-HT biosynthesis pathway and thus, may need to maintain a high level of unedited *5-HT$_{2C}$R* mRNAs to compensate for their naturally low concentration of 5-HT. These observations imply that editing responsiveness to stress and medication is modulated by genetic background, as well as behavioral state, suggesting that association studies using postmortem brains have limitations and that the development of animal models, such as 5-HT$_{2C}$R editing-deficient mice, will be needed to gain substantial insights into the relationships between RNA editing of 5-HTRs and mental disorders.

6. Involvement of ADARs in hypermutation and persistent infection of measles virus

A-to-I RNA editing seems to be responsible for several reported cases of biased hypermutation affecting RNA viruses during persistent infections in humans that lead to severe neurological disease [18]. In the matrix gene of a defective measles virus, isolated from the brain tissue of a measles inclusion body encephalitis (MIBE) patient, 50% of the A residues (132 of 266A) of the minus-strand RNA genome were found to be mutated to G after cDNA cloning and sequencing of the viral RNAs. These mutations in the viral RNAs appear to have been introduced by ADARs [18,87]. The extensive sequence changes drastically altered the coding capacity of the matrix gene including the elimination of a translation initiation codon, which resulted in suppression of translation of its mRNA [18]. The lack of matrix gene expression may help the virus to escape immune system recognition and thus, allow persistent infection [18]. Two more examples of highly biased A to G mutations have been found in the hemagglutinin gene of another strain of measles virus isolated from a patient with subacute sclerosing panencephalitis (SSPE) [18]. It has been speculated that biased hypermutation mediated by ADARs takes place *in vivo* as frequently in lytic as in persistent infections, but are propagated only in the genome of persistent viruses or defective-interfering particles because of the lower selective pressures operating in these infections. The frequency and extent of biased hypermutation events seen in persistant infections of the brain might also be a function of the high expression levels of ADAR1 and ADAR2 in brain [87].

7. *Outlook*

Changes in A-to-I RNA editing have now been linked to various human disorders and the increased understanding of the editing mechanism and target spectrum can explain some of the phenotypic features that result from RNA editing deficiency or hyperactivity. In general, a disease-related alteration of editing could manifest itself on several levels. If it is the editing substrate that becomes altered or misregulated the change in editing levels will be gene-specific and represent a secondary event in disease development. As a result, the editing efficiency and/or site-selectivity for this single substrate may be altered in all tissues where it is expressed. On the other hand, the aberration in RNA editing can be cell-type specific through deregulation of the editing machinery. Thereby, it may affect many transcripts within a single tissue or cell-type. The specific editing deficiency observed in motor-neurons of sporadic ALS patients [34,54-56] is an example for such a scenario as well as the hyperediting phenotype seen within immune cells during inflammation [88]. Lastly, the editing hyper- or hypoactivity of an ADAR enzyme may be global, thereby affecting many different RNAs with diverse consequences.

In the future, animal models that mimic individual situations will be the most appropriate means of studying the causal relationships between RNA editing malfunction and disease phenotypes. It is crucial to understand how A-to-I editing is regulated *in vivo* – a topic that is only beginning to be investigated. Important observations regarding this aspect are that ADARs are expressed from alternative promoters and undergo various alternative splicing events [89-91], the activity of ADAR enzymes is modulated by post translational modification [92], regulation of subcellular localization of ADARs [93-96], and a small molecule (inositol hexakisphosphate, IP6) was recently recognized as an essential co-factor for ADAR catalytic function [97]. ADAR activity is also affected by the action of small nucleolar RNAs (ADAR2) [98] and through alternative dsRNA substrate conformation (ADAR1) [99].

What makes studying these interrelationships more difficult and at the same time more interesting is the realization that RNA editing is closely linked to other RNA processing events as well as regulatory mechanisms mediated by RNA molecules. It has been recently reported that miRNA precursors are also subject to A-to-I RNA editing [100-102], and the miRNA biogenesis pathway and the expression levels of mature miRNAs are regulated by A-to-I RNA editing [100]. Interestingly, the cytoplasmic, full-length isoform of ADAR1p150 sequesters siRNA and suppresses the potency of siRNA [47]. Tudor-SN, a component of RISC (RNA-induced silencing complex), cleaves specifically highly edited dsRNAs with continuous I·U and U·I base pairs [103,104]. Finally, the chemotaxis defective phenotype of *C. elegans* strains containing homozygous deletions of *c.e.ADA1* and *c.e.ADA2* revealed that their phenotype can be reverted through inactivation of the RNAi pathway [105]. These recent findings all indicate frequent interactions between A-to-I editing and RNA silencing pathways and a potential role for ADARs in the regulation of RNAi. It should be pointed out that malfunctioning of the editing mechanism and consequent deficiencies in editing of protein coding and non-coding RNAs as well as insufficiency in the interaction between the RNAi and editing pathways may give rise to human diseases and pathophysiology much more complicated than those caused by mutation of a single protein coding gene.

Abbreviations

$5HT_{2C}$	serotonin receptor subunit 2C
ADAR	adenosine deaminase acting on RNA
ALS	amyotrophic lateral sclerosis
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
DSH	dyschromatosis symmetrica hereditaria
EAAT2	excitatory amino acid transporter 2
GluR	glutamate receptor
RISC	RNA-induced silencing complex
SOD1	superoxide dismutase 1

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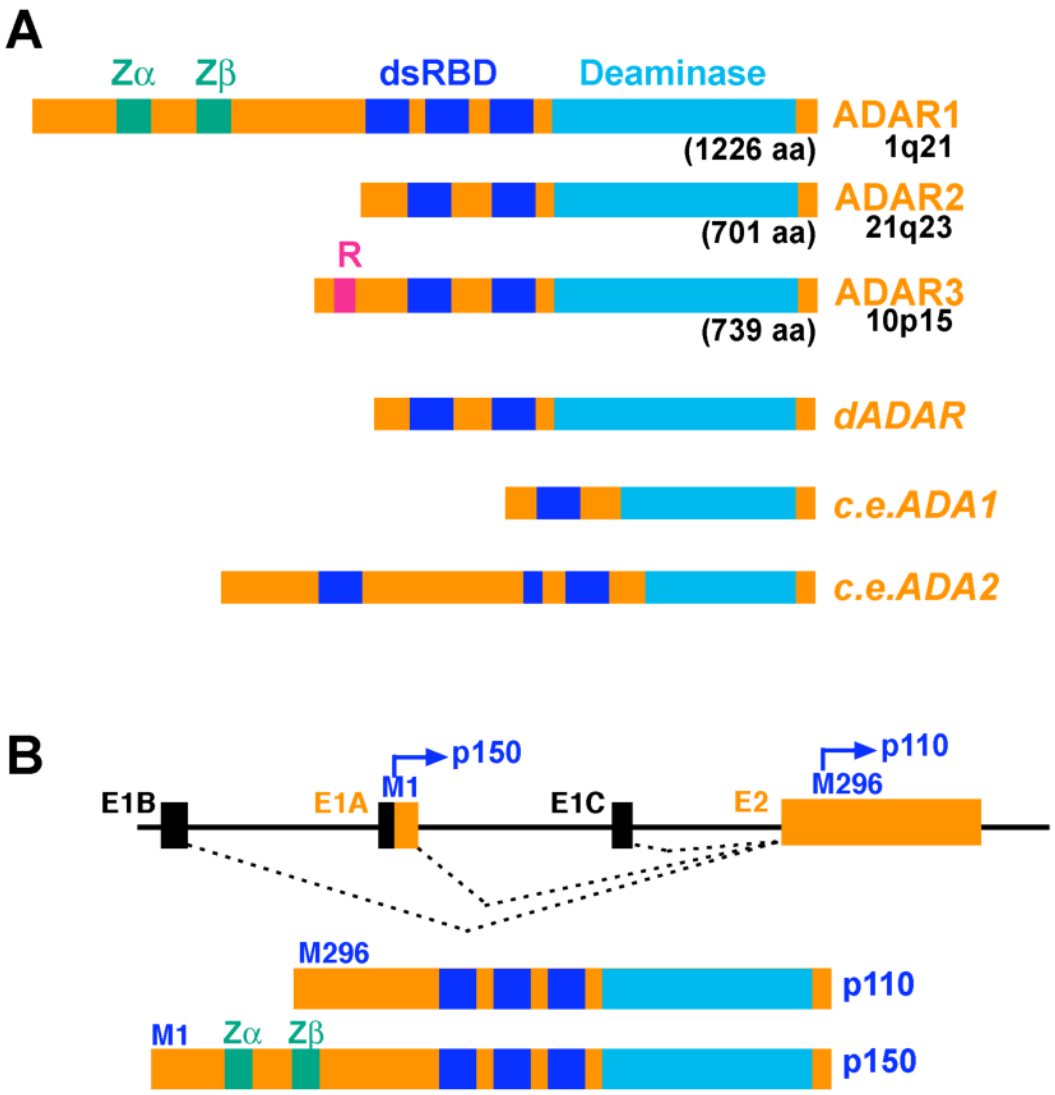

Figure 1. Members of the ADAR gene family

A) The structure of vertebrate and invertebrate ADAR gene family members. Indicated are two Z-DNA binding domains (ADAR1), the dsRNA binding (dsRBDs), the arginine-rich (R-domain), ssRNA binding domain (ADAR3) and the deaminase domain. For human ADAR1-3 amino acid lengths and chromosomal localization are shown. The structure of *Drosophila* dADAR and two *C. elegans c.e.ADA1* and *c.e.ADA2* are shown. *Drosophila dADAR* is very similar to mammalian ADAR2. *c.e.ADA1* contains a single copy of a poorly conserved dsRBD and a conserved deaminase domain, whereas *c.e.ADA2* contains three copies of dsRBDs and a deaminase domain that is rather divergent from any known ADAR genes. **B)** Two ADAR1 translation products. Exons E1A (IFN/dsRNA-inducible) and E1B and E1C (constitutive) are spliced to exon 2 at precisely the same junction. Exon 1A contains the Met initiation codon for the 1226-aa ORF specifying the IFN/dsRNA-inducible p150 protein. Exon 1B and 1C do not contain an AUG initiation codon. The second AUG initiation codon located in exon 2 initiates translation of the 931-aa encoding the constitutively expressed ADAR1 p110 protein.

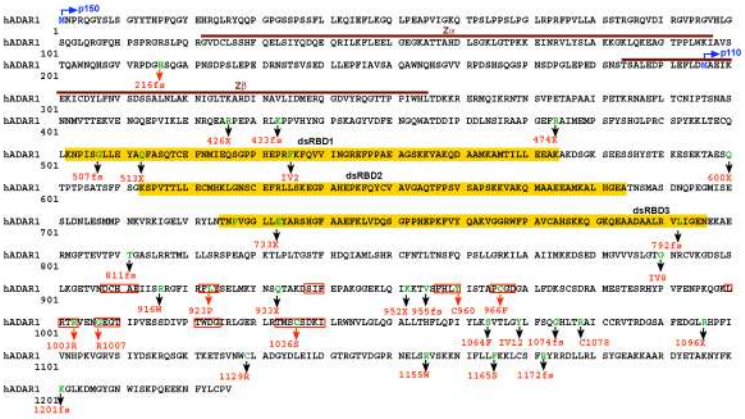

Figure 2. Mutations of the ADAR1 gene identified in patients with DSH

Total of 32 mutations (wild-type, green; mutation, red) associated with DSH have been identified in Japanese and Chinese populations [35-44]. Indicated are Zα and Zβ domains (brown overline), three dsRNA binding domains dsRBD1-3 (yellow filled box), and nine stretches of core deaminase subdomains, highly conserved in all three mammalian ADAR gene family members ADAR1-3 (red box). Two methionines (M1 and M296) used for alternative translation initiation of p150 and p110, respectively, are highlighted in blue.

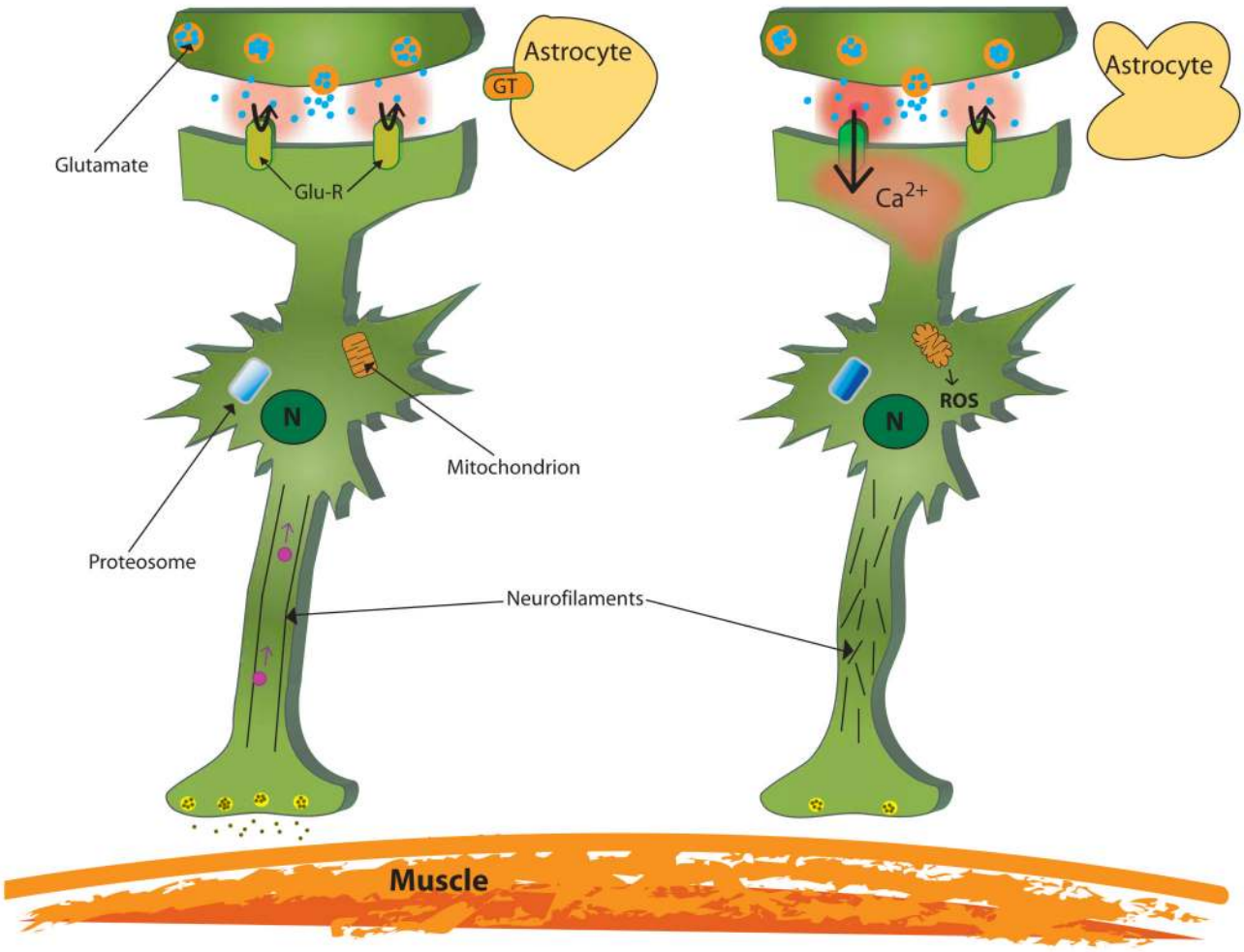

Figure 3. Contribution of RNA editing deficiency to motor neuron toxicity in ALS

The specific reduction in glutamate receptor GluR-2 Q/R site editing in ALS motor neurons leads to excessive influx of Ca^{2+}-ions through unedited glutamate gated channels (right panel). In concert with other molecular events that increase glutamate toxicity (glutamate transporter deficiency) motor neurons a subjected to increased stress that leads to accumulation of radical oxygen species (ROS), protein degradation dysfunction, neurofilament entanglement and eventually, neuronal death. N= nucleus; GT=glutamate transporter

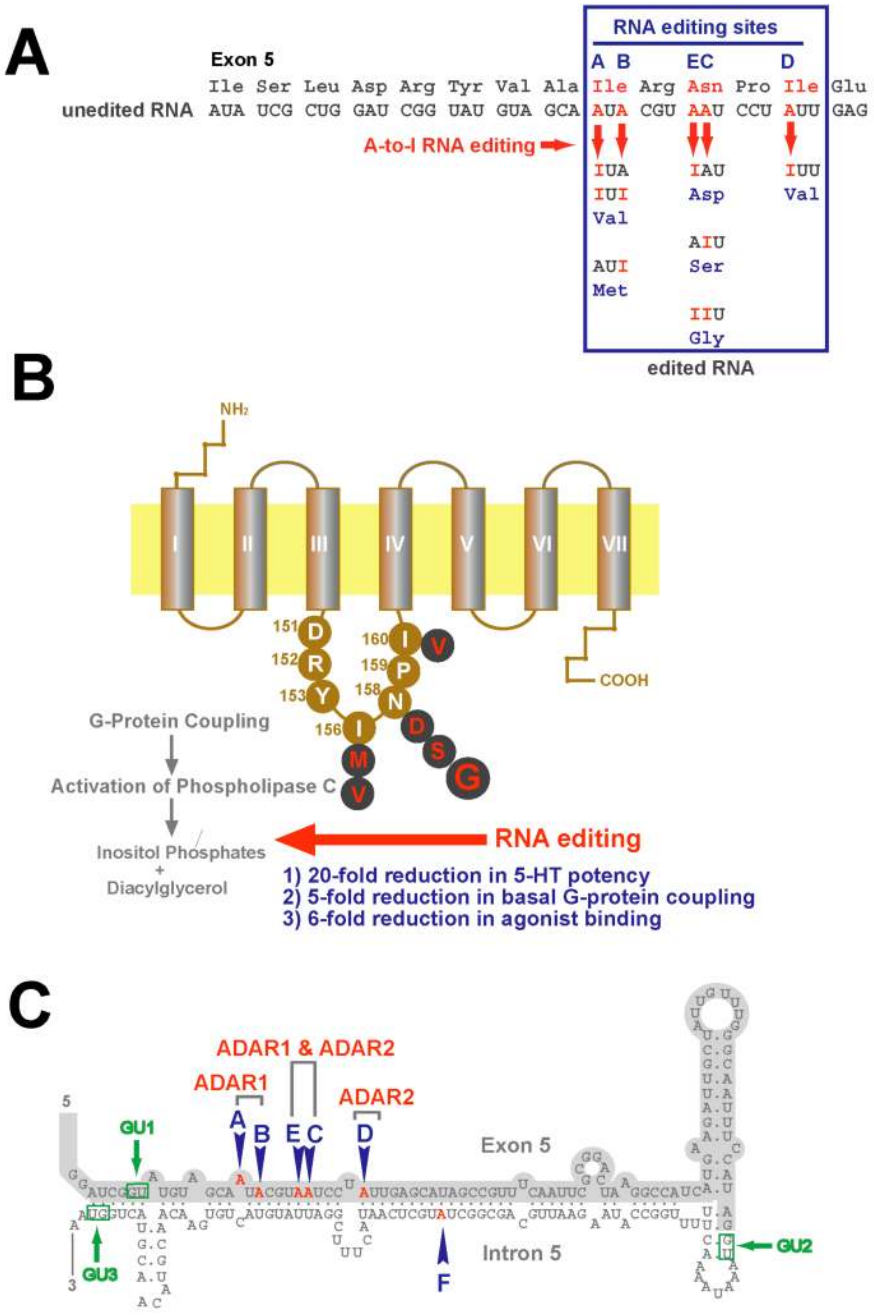

Figure 4. RNA editing of 5-HT$_{2C}$R by ADARs can generate various protein isoforms with altered functions

A; Genomic DNA and aligned amino acid sequence of the 5-HT$_{2C}$R intracellular loop II region. Five RNA editing sites detected (A-E sites) within exon 5 are shown in red. A combination of A-to-I RNA editing events at these sites (detected as A-to-G cDNA sequence change) result in various amino acid sequence alterations (*open box*).

B; The seven-transmembrane receptor 5-HT$_{2C}$R (*dark gray cylinders with loops*) is shown embedded within the lipid bilayer (*lighter yellow in the background*). Seven amino acids (*white characters in brown circles*) in the intracellular loop II region important for G-protein coupling are depicted together with substitutions brought on by editing (*red characters in black*

circles) at these locations. The unedited protein isoform is denoted as "INI" indicating the amino acids specified by the unedited codons. The fully edited isoform transforming "INI" to "VGV" profoundly reduces 5-HT potency, G-protein coupling and agonist binding, thereby affecting downstream signaling pathways. Among the edited 5-HT_{2C}R isoforms, the asparagine to glycine change at position 158 causes the most profound alteration in function and is therefore shown with slightly larger size.

C; Formation of a RNA fold back structure involving exon 5 (*lightly shaded*)/intron 5 of the human 5-HT_{2C}R gene transcript is necessary for site-selective editing at the A-E sites by ADARs as well as for intronic F site editing. Editing of the A and B sites is preferentially catalyzed by ADAR1 and the D site by ADAR2. The E and C sites may be edited efficiently by both ADAR1 and ADAR2. Among three alternative splicing donor sites (*GU1 to GU3 in green*), only GU2 leads to the mature mRNA that produces functional, full-length 5-HT_{2C}R protein. The editing pattern modulates splice site selection.

Table 1

The pattern of 5-HT_{2C}R editing in subjects with psychiatric disorders

Reference	Diagnosis	Brain area examined	N[a]	RNA editing status and/or pattern in specimen compared to controls	Other observations
Sodhi et al (82)	Schizophrenia	Frontal cortex	5	Decreased editing of B site	Increased expression of the unedited isoforms
Dracheva et al. (79)	Schizophrenia	Prefrontal cortex	15	No alteration	
Gurevich et al. (83)	Major depression	Prefrontal cortex	6	Increased editing of E site Decreased editing of D site Trend of increased editing at C site	All the subjects were suicide victims
Niswender et al. (80)	Schizophrenia	Prefrontal cortex	13	No alteration	Increased editing of A site in suicide subjects
	Major depression	Prefrontal cortex	13	No alteration	
Iwamoto et al. (81)	Schizophrenia	Prefrontal cortex	13	No alteration	Trends for increased editing of A site in suicide subjects
	Bipolar disorder	Prefrontal cortex	12	No alteration	
	Major depression	Prefrontal cortex	11	Trend of increased editing at D site	

[a]The number of subjects